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Prussic Acid in Burma Beans

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Prussic Acid in Burma Beans.

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Introductory.

THIS question was first raised in a letter received by the Director of Agriculture from the Director of the Imperial Institute who pointed out that the poisonous character of occasional cargoes of Burma beans had been noticed by London importing firms.

Analyses of beans from various sources were quoted in this letter and the suggestion made that the Department might encourage the cultivation of varieties freer from hydrogen cyanide than *Phaseolus lunatus*.

This letter was considered at a Departmental Conference in 1912 when the following lines of work were decided upon :—

1. Importation of Madagascar beans to be grown here and tested by the Imperial Institute.
2. Collection of all bean varieties grown in the province for submission of samples to the Imperial Institute.

On his return from leave the Agricultural Chemist proposed to undertake work on the prussic acid content of the commonest Burma bean *Pe-gya*. This was at the time not approved of, but a Conference held a year later raised no objection and accordingly work was commenced.

The work on the Madagascar bean has been carried on in the meantime and progress on it has been regularly reported in the annual report of the Mandalay Agricultural Station.

The conclusions arrived at up to the present are :—

1. The cultures so far examined are not suitable agriculturally to replace *Pe-gya* and *Pe-byu-gale*.
2. Prussic acid determinations showed an increase of poison for two years from 0·0025 to 0·008. Next year, however, the figure dropped to 0·004.

These differences were attributed to variations in climatic conditions. We have, however, no real clue to the manner in which the climate affects the poison content.

In any case, the Madagascar bean has not solved our difficulties. Its hydrogen cyanide content has increased and the plant has been found to be agriculturally unsuitable to replace the beans which it was intended to replace. Analyses of selected red and white beans grown

by the Department were also made by the Imperial Institute. The figures showed great variations. Generally speaking the red beans were the worst and the white beans worse than the Madagascar bean.

The results with the Burma beans were of course expected but the results with the Madagascar bean were particularly discouraging as they seemed to show that our climate and soil conditions tended invariably to maintain the hydrogen cyanide content of *Phaseolus lunatus* beans at a high level.

The present enquiry based on a search for non-poisonous cultures from the ordinary Burmese crop of *Pe-gya*, has yielded more encouraging results.

Analytical Processes.

In commencing this work the following recognized method was first tested :—

The sample to be analysed is ground fine and extracted with alcohol until all the glucoside has been removed.

The alcoholic liquid containing the glucoside is evaporated, taken up with water hydrolized with dilute acid and the hydrogen cyanide distilled off and absorbed into sodium bicarbonate solution.

The hydrogen cyanide in this liquid is determined by iodine titration. The first two modifications found to be necessary were :—

(*a*) *Improved distillation.* This was effected by allowing a slow current of air to bubble through the hydrolized liquid heated in a water bath.

(*b*) *Absorption of hydrogen cyanide.* By distilling hydrogen cyanide solutions of known strength it was found that absorption in sodium bicarbonate solution was generally far from complete. An absorption tower of glass beads was therefore arranged. This gave perfect satisfaction with potassium cyanide solutions of known strength.

The next point noted was that successive distillates from bean extract continued to give a slight iodine reaction for a long time after the prussic acid had been entirely removed.

This has been proved over and over again and must be accepted as quite certain. That it is not due, for instance, to the presence of traces of sulphurous acid when hydrolysis is effected with sulphuric acid is proved by the fact that the identical difficulty is encountered if hydrolysis is effected with hydrochloric acid.

In the case of this bean therefore the iodine titration cannot be relied upon to give a true figure for hydrogen cyanide content. An

attempt was made to improve conditions by altering the strength of acid used for hydrolysis.

This had little or no effect on the reduction of iodine absorbing substances other than hydrogen cyanide but materially reduced the rate of liberation of prussic acid. Nothing was to be gained in this way therefore.

A series of experiments was then undertaken to determine how far the formation of Prussian Blue could be used for the estimation of hydrogen cyanide.

This reaction has been used by a number of workers for this purpose, but none of the methods described was applicable in the present case. The action had, for reasons which need not be entered into, to take place in about 400 c.c. liquid containing sodium hydrogen carbonate and had preferably to take place without the aid of heat.

Starting with these conditions a large number of tests were made. Of these only the following need be quoted here :—

1. Effect of quantity of ferrous sulphate used on yield of Prussian Blue. The following experiment was made with 2 c.c. and 5 c.c. potassium cyanide solutions always made up to 400 c.c.

In each case all other factors remaining the same the amount of ferrous sulphate was varied. The Prussian Blue formed was filtered, ignited and weighed as (Fe_2O_3) ferric oxide.

10 per cent. $FeSO_4$ (ferrous sulphate)	Ferric oxide (Fe_2O_3) obtained	
	using 2 c.c. KCN (Potassium cyanide)	using 5 c.c. KCN (Potassium cyanide)
4 c.c.	0·0014	0·0036
8 c.c.	0·0018	0·0050
16 c.c.	0·0022	0·0054
24 c.c.	0·0022	0·0054

These figures show that quantities up to 8 c.c. of ferrous sulphate solution were not sufficient to obtain maximum yield of Prussian Blue.

For all subsequent determinations 16 c.c. of ferrous sulphate solution were used. It need scarcely be remarked that this test had to be repeated numbers of times with other conditions also varied before optimum results were obtained.

2. Effect of alkali on yield of Prussian Blue. It was noticed that in certain cases, the presence of sodium hydrogen carbonate may entirely prevent the formation of Prussian Blue.

Various proportions of alkali hydrate were added to the liquid and were found to have an important effect on the yield of Prussian Blue. The point is of interest and deserves attention.

The following set of results will indicate the effect of the nature of the alkali upon the result. The experiment was made with three strengths of potassium cyanide, in every case using 16 c.c. of ferrous sulphate solution, shaking for 3 hours and acidifying. The Prussian Blue was determined after reprecipitation, filtration and ignition as (Fe_2O_3) ferric oxide.

Alkali used		Ferric oxide (Fe_2O_3) obtained		
Normal Pot. Hydroxide	Normal Bicarbonate	using 5 c.c. KCN (Potassium cyanide)	using 2 c.c. KCN (Potassium cyanide)	using 0·5 c.c. KCN (Potassium cyanide)
0	60	0·0044	0·0014	0·0002
10	50	0·0012	0·0016	0·0004
20	40	0·0050	0·0020	0·0004
30	30	0·0052	0·0020	0·0004
40	20	0·0044	0·0010	0·0000
50	10	0·0042	0·0008	0·0000
60	0	0·0038	0·0006	0·0000

These figures show clearly that to obtain maximum yields, it is essential to adjust the proportion of alkali and carbonate to be approximately equal to normal carbonate.

In fact, when dealing with small quantities of hydrogen cyanide, a weighable quantity of Prussian Blue cannot be obtained unless this adjustment is made.

In the case of 0·5 c.c. potassium cyanide in the table above, for example, where zero weights are given, there was actually no colour visible whilst using 10, 20 and 30 c.c. of potassium hydroxide an intensely coloured precipitate of Prussian Blue was obtained. It should be stated here that the filter paper invariably absorbs a certain amount of the soluble salts. In a very large number of blank determinations the ash was found to weigh 0·0004 gm. with perfect constancy. This correction has been made in all the above and in subsequent weighings.

There is little doubt that the effect of the nature of the alkali used is due in part to the nature of the ferrous oxide precipitate formed.

When potassium hydroxide is in excess, the green voluminous hydroxide originally precipitated very soon turns black, shrinks and forms a relatively granular precipitate.

With the normal carbonate a dark blue-green voluminous precipitate is formed which remains unaltered and obviously offers a large reaction surface to the hydrogen cyanide in solution.

3. The process adopted for determination of hydrogen cyanide. As a result of a number of experiments of which two series have been cited, the following process was adopted.

The liquid containing hydrogen cyanide + 30 c.c. sodium hydrogen carbonate made up to between 400 and 500 c.c. in volume is treated with 30 c.c. of normal potassium hydroxide, 16 c.c. ferrous sulphate solution are added and the flask shaken at regular intervals for three hours.

The liquid is then acidified and allowed to stand some days for the Prussian Blue to settle out. The precipitate is then filtered, taken up with a small amount of alkali and the Prussian Blue reprecipitated in a small volume of liquid.

If the amount of Prussian Blue is small, it is determined by colour comparison with standards, if large it is filtered, ignited and weighed as Fe_2O_3 (Ferric oxide).

The Prussian Blue determinations described in the two previous series of experiments were made in this way with the modifications specified in each particular case.

By the method just described, the following results were obtained with different quantities of potassiam cyanide, the liquid in each case being diluted to 500 c.c.

Potassium cyanide	Ferric oxide obtained	
	(1)	(2)
0·2	0·0000	0·0000
0·5	0·0004	0·0004
1·0	0·0010	0·0010
2·0	0·0020	0·0022
5·0	0·0052	0·0054
10·0	0·0108	0·0108

The determinations were made in duplicate. The first set was exactly as described. In the second set the crude ferrocyanide was treated with ammonium chloride which is unnecessary in this case but with bean extracts helps to eliminate organic matter. The strength of the potassium cyanide solution used in this test (as determined both by standard iodine and standard silver nitrate) was 0·000938 gm. hydrogen cyanide per c.c.

Ten c.c. of this liquid completely converted to Prussian Blue and ignited should yield theoretically 0·0108 gm. ferric oxide, *i.e.*, the identical figure obtained above.

The precipitation in this case therefore appears to be absolutely quantitative. It may be noticed however that with very dilute solutions undoubted loss occurs.

This may be due either to incomplete reaction or to incomplete precipitation. The limit of quantitative precipitation is reached with 1 c.c. of potassium cyanide solution. Below this amount and down to 0·2 c.c., colorations can be obtained.

The lowest quantity of hydrogen cyanide which has been detected is that contained in 0·2 c.c., *i.e.*, 0·000047 gm. in 500 c.c. liquid or roughly 0·0000001 gm. hydrogen cyanide per c.c.

This is a very favourable result compared with the figures obtained by Anderson (*Journal of the Society of Chemical Industry*, 1916, page 1083).

In our case however relatively very large volumes of liquid were employed and the colours then concentrated by reprecipitation. There is no doubt, however, that the favourable results are mainly due to the discovery that the action is more perfect when the alkalinity of the liquid is carefully adjusted.

The process as worked out and described here should obviously be most effective in determining the small quantities of hydrogen cyanide present in beans. It was remarked earlier that alcoholic extracts of *Pe-gya* when hydrolized by dilute acids continued to evolve substances which reacted with iodine after all the hydrogen cyanide had been distilled off. This was very easily proved by the above Prussian Blue method.

A third fraction distilled from a bean extract was divided into three parts. One part was titrated and gave an appreciable iodine absorption figure. The second part was treated for Prussian Blue reaction and gave no trace of colour. To the third part 0·2 c.c. potassium cyanide was added. This liquid on treatment gave the Prussian Blue reaction.

The tests show that the original liquid though it gave an iodine reaction contained no measurable amount of hydrogen cyanide.

Prussic acid in Pe-gya cultures.

About 100 single plant samples were collected from cultivators' fields from the typical *Pe-gya* growing areas in Sagaing District.

These were all grown separately and analysis of the crops commenced ; the process employed being the Prussian Blue method already described.

When the next sowing season arrived, all the cultures had not been tested and it was decided therefore to confine the enquiry in the first place to those cultures which had been examined. The prussic acid content of these ranged from 0·0004 per cent. to 0·03 per cent.

From these cultures the two best, the two worst and four intermediate specimens were selected for further work. From their appearance absolutely no distinction was possible between the samples. They all consisted of a mixture of distinctly mottled with some more uniformly brown-coloured beans.

This brown colour is known to develop gradually on keeping. All the beans become darker in time, but with some the colour change is more rapid than with others.

It seemed desirable to find out whether this colour difference to be found within each single plant culture was in any way connected with the prussic acid content.

To test this point, the two best and the two worst cultures were each divided into two sub-samples consisting of—

(*a*) distinctly mottled seed ;

(*b*) more uniformly brown-coloured seed.

There could be no doubt that if prussic acid content were connected with the observed colour differences, this separation though obviously far from perfect would give positive results in the succeeding crop.

The following table shows the prussic acid content of the cultures used :—

Sample	Hydrogen cyanide content
1. Mottled seed	1 and 2 mixed 0·0004
2. Brown seed	
3. Mottled seed	3 and 4 mixed 0·0012
4. Brown seed	
5. Mixed	.... 0·0018
6. Mixed	.... 0·0022
7. Mixed	.... 0·0030
8. Mixed	.... 0·0112
9. Mottled seed	9 and 10 mixed 0·0138
10. Brown seed	
11. Mottled seed	11 and 12 mixed 0·0347
12. Brown seed	

NOTE. Nos. 1 and 2 were mottled and brown seed obtained from a single plant culture which gave average hydrogen cyanide content 0·0004. The same remark applied to Nos. 3 and 4, Nos. 9 and 10, and Nos. 11 and 12.

These seeds were grown at three different stations during the past season. The places selected were—

(1) Mandalay—in the dry zone.
(2) Hmawbi—in the wet delta zone.
(3) Tatkon—in middle Burma where the rainfall is intermediate in character.

At each station the cultures were grown in parallel rows of 20 plants, the whole series being duplicated.

The sowing season was in each case unfavourable and germination poor especially at Hmawbi.

Some cultures from which only a few plants grew up were not examined. For Hmawbi and Tatkon the crops from the two identically numbered rows were combined to yield one sample and analysed mixed.

At Mandalay, the crop from each row was analysed separately and thus duplicate figures obtained to verify the results.

Further at Mandalay sowings were made at three different dates. From these the effect of sowing season upon hydrogen cyanide content can be estimated.

The following results were obtained :—

Hydrogen cyanide in *Pe-gya* crops from selected cultures.

	Mandalay			Hmawbi	Tatkon
	1st sowing	2nd sowing	3rd sowing		
1 . . .	..	..	0·0008	..	0·0010
2 . . .	0·0008	0·0012	0·0010	0·0015	0·0015
3 . . .	..	0·0016	0·0015	..	0·0016
4 . . .	0·0016	0.0021	..	0·0017	0.0016
5 . . .	0·0016	0·0021	0·0021	..	0·0021
6 . . .	0·0021	0·0027	0·0026	0·0027	0·0024
7 . . .	0·0028	0·0043	0·0062	0·0036	0·0036
8 . . .	0·0058	0·0072	0·0146	0·0094	0·0043
9 . . .	..	0·0249	..	..	0·0108
10 . . .	0·0083	0·0242	..	0·0220	0·0101
11 . . .	..	0·0317	0·0317	..	0·0180
12 . . .	0·0250	0·0314	0·0325	0·0311	0·0188

In spite of some unavoidable blanks, the table of results is sufficiently complete to show conclusively that whatever soil, climate or season these cultures were grown in, a steady increase of hydrogen cyanide was obtained on passing down the series.

This is to say the seed which contained least hydrogen cyanide last year again contains the least when grown under a variety of conditions. Similarly the seed with most hydrogen cyanide last year is found to contain most this year.

In other words the cultures have maintained the property of producing the same relative amounts of poison. As this has been shown to be the case under almost the greatest extremes of environment obtainable in the Province, there can be no doubt left that the content of hydrogen cyanide is a characteristic property of each one of cultures which has been separated out. As long as mixing is prevented the cultures must maintain these differences.

We therefore are in possession of cultures which contain quite harmless amounts of hydrogen cyanide and we know they will breed true in this respect.

It remains still to consider how far the effects of soil and climate may counteract this satisfactory conclusion. That the climate can very considerably modify the amount of hydrogen cyanide is seen from the results of the three sowings at Mandalay. The general superiority of the early crop is in this case very marked. It is of course impossible at present to say that the early crop will always be superior in this respect to the later sowings. This point is now being studied. It is certain however that marked differences are to be expected.

That the combined effects of soil and climate can also cause considerable variations in hydrogen cyanide content of the crop is seen by comparing the Hmawbi and Tatkon results. The Hmawbi figures were for this particular year much higher than those of Tatkon. The effect of the soil is probably considerable and is being studied more fully.

The points so far considered certainly prove without doubt that from a cultivator's ordinary mixed crop of seed, great variations in the content of hydrogen cyanide are to be expected under different conditions of growth. An examination of the table above, however, shows that this is not necessarily the case with our best selected cultures.

It will be noticed that Nos. 1 and 2 give practically the same values under all the conditions tested. The figures in this case require to be more fully investigated by working on a larger scale to bring out small differences that we must expect from variation of environment. The

results obtained so far however show that the hydrogen cyanide in these cultures (whether it does or does not appreciably vary with varying environment) remains very low under each of the extreme conditions tested.

Exceptionally unfavourable conditions may of course exist and therefore the main bean-producing areas should be tested by the above methods.

It was explained earlier that the pairs of samples 1 and 2, 3 and 4, 9 and 10; 11 and 12 were really four main and distinct cultures each of which had been divided into two parts according to slight colour variation in the seeds.

The results for these samples are brought together here :—

	1	2	3	4	9	10	11	12
Mandalay	0·0008	0·0010	0·0016	0·0021	0·0249	0·0242	0·0317	0·0314
Tatkon .	0·0010	0·0015	0·0016	0·0016	0·0108	0·0101	0·0180	0·0188

These figures show the effect of environment already referred to. For example Nos. 9 and 10 grown at Mandalay give much higher figures than when grown at Tatkon.

The point to note however is that Nos. 9 and 10 grown at Mandalay give practically identical figures. Under Tatkon conditions, the hydrogen cyanide content of these two samples is notably altered but the alteration is the same for both samples. These remarks apply equally well to the samples 11 and 12 derived from another main culture.

These results prove that the colour differences observed within each of our main cultures do not indicate differences in hydrogen cyanide production by the progeny of these seeds. In other words cultures which are pure as regards the production of hydrogen cyanide may contain seeds which differ from one another slightly in colour.

CONCLUSIONS.

The results obtained in this work may be summarized as follows :—

1. The content of hydrogen cyanide is an inherited character of pure single plant cultures. These cultures may be multiplied and will maintain the differences noted.

2. The hydrogen cyanide present in the cultures is found to vary considerably according to soil and climatic conditions.
3. Cultures giving low amounts of hydrogen cyanide in one locality give low figures under all the conditions tested.
4. Differences in colour in seeds from a single culture do not indicate differences in the power of producing hydrogen cyanide in their progeny.
5. The best cultures so far found always contain some hydrogen cyanide. But the quantity is only half that contained in the original sample of Madagascar bean imported into the Province as safe.

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